

WHAT IS CLAIMED IS:

1. A method for producing linearly amplified amounts of antisense RNA from mRNA, said method comprising:
- 5 (a) converting mRNA to double-stranded cDNA, wherein one terminus of said double-stranded cDNA comprises an RNA polymerase promoter region; and
- (b) transcribing said double-stranded cDNA into antisense RNA in the presence of a reverse transcriptase that is incapable of RNA-dependent DNA polymerase activity during said transcribing step.
- 10 2. The method according to Claim 1, wherein said method further comprises inactivating said reverse transcriptase prior to said transcribing step.
3. The method according to Claim 2, wherein said inactivation is accomplished
- 15 by heating the reaction mixture.
4. The method according to Claim 1, wherein said method further comprises inhibiting said reverse transcriptase with an inhibitor during said transcribing step.
- 20 5. The method according to Claim 4, wherein said inhibitor is at least one ddNTP.
6. The method according to Claim 1, wherein said converting step comprises a single cDNA synthesis step, wherein the same polymerase is employed for the synthesis of first and second cDNA strands.
- 25 7. The method according to Claim 1, wherein said converting step comprises a first strand cDNA synthesis step and a second strand cDNA synthesis step.
8. The method according to Claim 7, wherein a first polymerase is employed for
- 30 synthesis of said first strand cDNA and a second polymerase is employed for synthesis of said second strand cDNA, wherein said first polymerase is lacking RNaseH activity.
9. The method according to Claim 1, wherein said converting step employs a promoter-primer comprising an mRNA binding site linked to a promoter sequence.

RNA.

16. The method according to Claim 15, wherein said RNA-dependent DNA polymerase activity, RNaseH activity and DNA-dependent DNA polymerase activity  
5 are contributed by a single polymerase.

17. The method according to Claim 16, wherein said polymerase is the reverse transcriptase of Moloney Murine leukemia virus (MMLV-RT).

10 18. The method according to Claim 16, wherein said polymerase is the reverse transcriptase of avian myeloblastosis virus (AMV-RT).

19. The method according to Claim 10, wherein said RNA-dependent DNA polymerase activity is inhibited with ddNTPs.

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20. A method for producing linearly amplified amounts of antisense RNA from mRNA, said method comprising:

(a) contacting mRNA with a promoter-primer in the presence of a first polymerase having RNA-dependent DNA polymerase activity and lacking RNaseH activity under  
20 conditions sufficient for first strand cDNA synthesis to occur to produce a hybrid of said mRNA and a first strand cDNA, wherein said promoter-primer comprises an mRNA binding site linked to a promoter sequence;

(b) contacting said hybrid with an enzyme catalyzing RNaseH activity under conditions sufficient to convert said complex to a double-stranded cDNA  
25 molecule; and

(c) transcribing said double-stranded cDNA into antisense RNA in the presence of ddNTPs; whereby said mRNA is linearly amplified into antisense RNA.

21. The method according to Claim 20, wherein said enzyme catalyzing RNaseH  
30 activity is the reverse transcriptase of Moloney Murine leukemia virus (MMLV-RT).

22. The method according to Claim 20, wherein said enzyme catalyzing RNaseH activity is the RNaseH of *Escherichia coli* (*E. coli* RNaseH).

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23. The method according to Claim 20, wherein said RNA polymerase promoter is the T7 promoter and said RNA polymerase is T7 RNA polymerase.

24. The method according to Claim 20, wherein said RNA polymerase promoter is the T3 promoter and said RNA polymerase is T3 RNA polymerase.

25. The method according to Claim 20, wherein said ddNTPs are selected from the group consisting of: ddATP and ddGTP.

26. A kit for use in linearly amplifying mRNA into antisense RNA, said kit comprising: an oligonucleotide promoter-primer comprising an RNA polymerase promoter sequence; and ddNTPs.

27. The kit according to Claim 26, wherein said kit further comprises at least one polymerase.

28. The kit according to Claim 26, wherein said polymerase is MMLV-RT.

29. The kit according to Claim 26, wherein said kit comprises a first RNaseH- polymerase and a second RNaseH+ polymerase.

30. The kit according to Claim 26, wherein said kit further comprises an RNA polymerase.

31. The kit according to Claim 26, wherein said RNA polymerase is T7 RNA polymerase.

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RNA.

16. The method according to Claim 15, wherein said RNA-dependent DNA polymerase activity, RNaseH activity and DNA-dependent DNA polymerase activity  
5 are contributed by a single polymerase.

17. The method according to Claim 16, wherein said polymerase is the reverse transcriptase of Moloney Murine leukemia virus (MMLV-RT).

10 18. The method according to Claim 16, wherein said polymerase is the reverse transcriptase of avian myeloblastosis virus (AMV-RT).

19. The method according to Claim 10, wherein said RNA-dependent DNA polymerase activity is inhibited with ddNTPs.

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20. A method for producing linearly amplified amounts of antisense RNA from mRNA, said method comprising:

(a) contacting mRNA with a promoter-primer in the presence of a first polymerase having RNA-dependent DNA polymerase activity and lacking RNaseH activity under  
20 conditions sufficient for first strand cDNA synthesis to occur to produce a hybrid of said mRNA and a first strand cDNA, wherein said promoter-primer comprises an mRNA binding site linked to a promoter sequence;

(b) contacting said hybrid with an enzyme catalyzing RNaseH activity under conditions sufficient to convert said complex to a double-stranded cDNA  
25 molecule; and

(c) transcribing said double-stranded cDNA into antisense RNA in the presence of ddNTPs; whereby said mRNA is linearly amplified into antisense RNA.

21. The method according to Claim 20, wherein said enzyme catalyzing RNaseH  
30 activity is the reverse transcriptase of Moloney Murine leukemia virus (MMLV-RT).

22. The method according to Claim 20, wherein said enzyme catalyzing RNaseH activity is the RNaseH of *Escherichia coli* (*E. coli* RNaseH).

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10. A method for producing linearly amplified amounts of RNA from mRNA, said method comprising:

(a) converting mRNA to cDNA with a promoter-primer comprising an mRNA binding site linked to a promoter sequence, wherein said cDNA comprises an RNA polymerase promoter region; and

(b) transcribing said cDNA into RNA in the presence of a reverse transcriptase that has been rendered ineffective for RNA-dependent DNA polymerase activity prior to said transcribing step.

11. The method according to Claim 10, wherein said method further comprises inactivating said reverse transcriptase prior to said transcribing step.

12. The method according to Claim 11, wherein said inactivation is accomplished by heating the reaction mixture.

13. The method according to Claim 10, wherein said method further comprises inhibiting said reverse transcriptase with an inhibitor during said transcribing step.

14. The method according to Claim 13, wherein said inhibitor is at least one ddNTP.

15. A method for producing linearly amplified amounts of antisense RNA from mRNA, said method comprising:

(a) converting mRNA to double-stranded cDNA, wherein one terminus of said double-stranded cDNA comprises an RNA polymerase promoter region by:

(i) contacting mRNA with a promoter-primer under conditions wherein said mRNA forms a complex with said promoter-primer, wherein said promoter-primer comprises an mRNA binding site linked to a promoter sequence; and

(ii) converting said complex to double-stranded cDNA using a combination of RNA-dependent DNA polymerase activity, RNaseH activity and DNA-dependent DNA polymerase activity; and

(b) transcribing said double-stranded cDNA into antisense RNA in the presence of a reverse transcriptase that is incapable of RNA-dependent DNA polymerase activity during said transcribing step; whereby said mRNA is linearly amplified into antisense

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